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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Assessment of axon health in spinal cord injury (SCI) is vital for proper diagnosis and treatment. Magnetic resonance imaging (MRI) is routinely performed in patients and provides valuable information about cord edema and hemorrhage. However, comprehensive prediction of axonal changes from in vivo MR imaging remains elusive. At the U. Penn site, we are applying two novel MRI methods to the problem of assessment of axonal loss, axonal diameter distribution, and myelin loss (q-space imaging (QSI) and ultra-short echo-time (UTE) MRI) first on animal specimens and then on human subjects.

During the final period of the project the focus of the research was on the core aim, which was to study Wallerian degeneration of spinal cord axons in a mouse injury model. Injury was induced by hemisection of the spinal cord (i.e. sectioning of only one hemisphere of the spinal cord, leaving the contralateral side intact, thereby providing an internal control). Spinal cords were examined after perfusion fixation by means of a quantitative magnetic resonance technique, called Q=space imaging or QSI, that provides indirect information on axonal structure by measuring diffusion of tissue water perpendicular to the spinal cord axis. Spinal cords were studied 3 weeks and 3 months post injury. Spinal cord degeneration was clearly evident and quantifiable at locations distant to the injury site in conformance with hypothesis. Finally, histologic images were obtained, which allow for direct visualization of axons. This portion of the project could not be completed yet but it is planned to finish the project provided funds can be obtained from other sources.

15. SUBJECT TERMS
Axon Architecture, Spinal Cord Injury, Axon Loss, Myelin, Q-Space Imaging, UTE, MRI

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Annual

Introduction - Objectives and Summary

The key objective of this project was to evaluate the axonal damage in a mouse spinal cord injury (SCI) model involving hemisection. Injury is expected to cause axonal degeneration. Sixteen mouse spinal cords were studied *ex vivo*. Of these, four were control specimens, and the remaining 12 were derived from mice undergoing a dorsal-lateral funiculotomy at the C-6 level. Six spinal cords were harvested three weeks after injury and three months after injury. Spinal cords were perfusion fixed and underwent Q-space imaging (QSI) at 400 MHz with a protocol similar to that described in our prior work (1,2) to yield diffusion displacement profiles which provide quantitative information on axonal structure indirectly, thus having potential for translation to humans with SCI. The plan then was to compare the QSI data with histology as ground truth. Completion of the project has been delayed due to the difficulties in generating the histologic sections and processing and analyzing the massive amount of digital histologic images, which required development of new algorithms for semi-automatic analysis of the data.

During the final year and following a no-cost extension, the project, though not yet completed, progressed in three areas: (1) Sectioning of fixed mouse spinal cords; (2) Development and testing of a new algorithm for segmentation of the histologic axon images; (3) Analysis of the QSI MR images.

Body/Key Research Accomplishments/ Reportable Outcomes

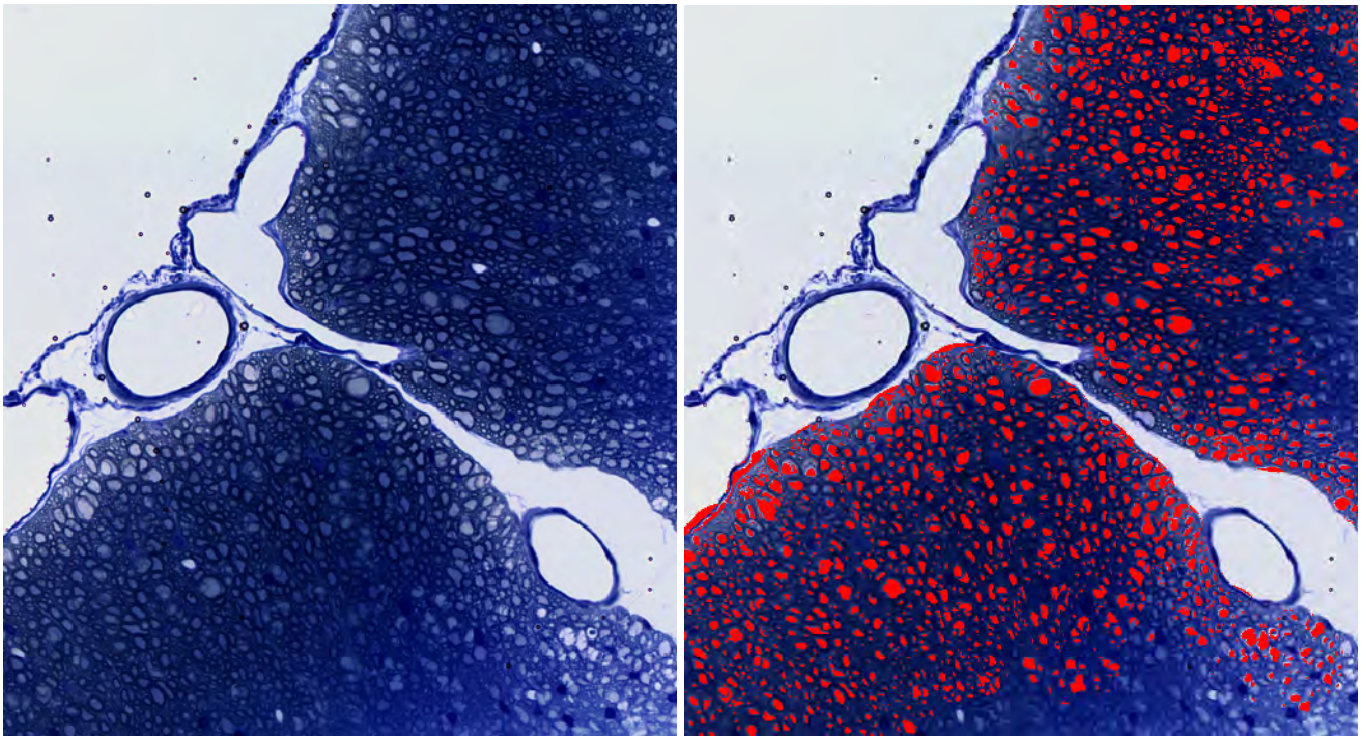
1. Sectioning of fixed mouse spinal cords

The Core Lab which did the pilot work is no longer available, which necessitated a search for a new resource (UPenn Electron Microscopy Core Lab) and required training of personnel to allow execution of the work within the scope of the limited residual funds. This work is now in progress. All mouse spinal cords have been embedded in resin prior to sectioning with a microtome. The embedded block portion is located from 2mm to 6mm from the rostral end of the cord. As determined on the basis of the MRI images, injuries are located approximately 4mm from the rostral end, placing them at the center of the resin blocks. Sections 1 micron thick are collected at roughly 250, 500, 1000, 1250, 1500, 1750, 2000, 2250, 2500, 2750, 3000, 3500 μm from the rostral end of the block (yielding on average 11 to 12 sections per sample). Each slide is then coverslipped with Permount and a thin plate of glass before being digitized. At this point we have completed sectioning of one spinal cord (sample #4, three months post-injury). Samples # 3, 5 (three weeks post-injury) and control #1 have been sectioned and are being coverslipped. We anticipate that the sectioning of the remaining spinal cords will be completed 7/2015.

2. Development and testing of a new algorithm for segmentation of the histologic axon images

We have conceived and implemented a semi-automated method for segmenting the axons on the basis of the histological images. Although the contrast is adequate between intra- and extra-axonal tissue, the main difficulty is the heterogeneity of the staining. Thus, using a simple threshold for the segmentation fails. As can be seen in Figure 1, the baseline intensity can change abruptly across only a few pixels, and therefore a local calibration (e.g., subtracting out the median signal within small regions) also fails. Another technique we attempted was to fit the baseline intensity to a polynomial in the X/Y spatial coordinates. This also did not work due to the high degree of non-linearity in the staining intensity.

We found an effective approach was to use a semi-automated segmentation software (developed in house) where the user drags a small ellipsoidal region across the image in a manner such that the staining intensity is roughly linear within the ellipse. As the user hold down and drags the mouse button, the software does the linear correction in real time and shows the segmentation in real time. Using keyboard arrows, the size and shape of the ellipse may be modified as well as the threshold level (which is applied after the linear correction). In this way the segmentation may be performed rapidly while avoiding situations where the ellipsoidal region contains a nonlinear staining gradient.



a b

Figure 1. A section of the histological image before (a) and after segmentation (b). Segmentation was performed with the semi-automated algorithm described above where the user drags a small ellipsoidal area across the regions of interest (ROI) in a manner that avoids locally nonlinear staining gradients.

3. Q-Space MR Imaging and Analysis

Q-space imaging (QSI) was performed on a total of 16 fixed mouse spinal cords. Spinal cords were first trimmed to remove the brain stem (to ensure that the specimen fit into a 5 mm NMR tube) and cut at the mid-thoracic level. A custom holder was constructed to hold a spinal cord inside a 5 mm NMR tube, which was then filled with Fomblin.

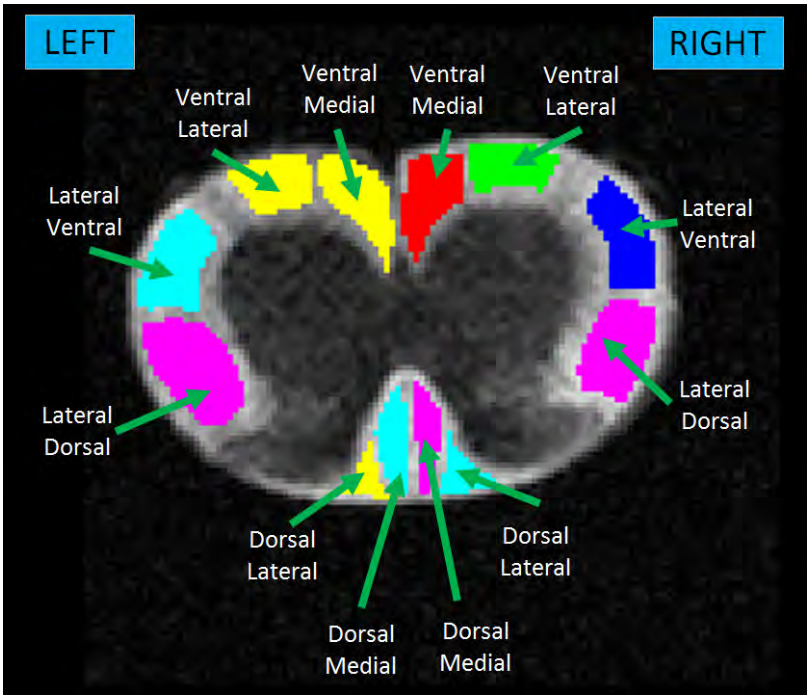


Figure 2. Representative manual ROIs overlaid on an axial diffusion-weighted image.

Based on a spin-echo T2-weighted image, the site of injury could be identified as a dark line. A 13 slice, 2D QSI experiment was performed on each spinal cord with the site of injury centered in slice 6. QSI processing was performed using a custom Matlab program to produce three parametric maps for each slice: full-width-at-half-maximum (FWHM), zero-displacement probability (ZDP), and kurtosis (1) (2).

ROIs were manually drawn as shown in Figure 2. The spinal cord was divided into left and

right halves and each halve was subdivided into six ROIs for a total of 12 ROIs. Dorsal-lateral funiculotomy was performed on the right side. With 13 MRI slices and 12 ROIs for each slice, a total of 156 ROIs were manually drawn. For each ROI, mean and standard deviation was recorded for all FWHM, ZDP, and kurtosis maps.

The unique aspect of this dataset is the ability to assess axonal injury at several distances both rostral and caudal from the site of injury as well as at different time points. As injury is only induced on the right side, the left side of the spinal cord can be used as a contralateral control. Figure 3 shows three-month and three-week FWHM, ZDP, and kurtosis values from the lateral dorsal ROI for each MRI slice. The values are averaged over all specimens.

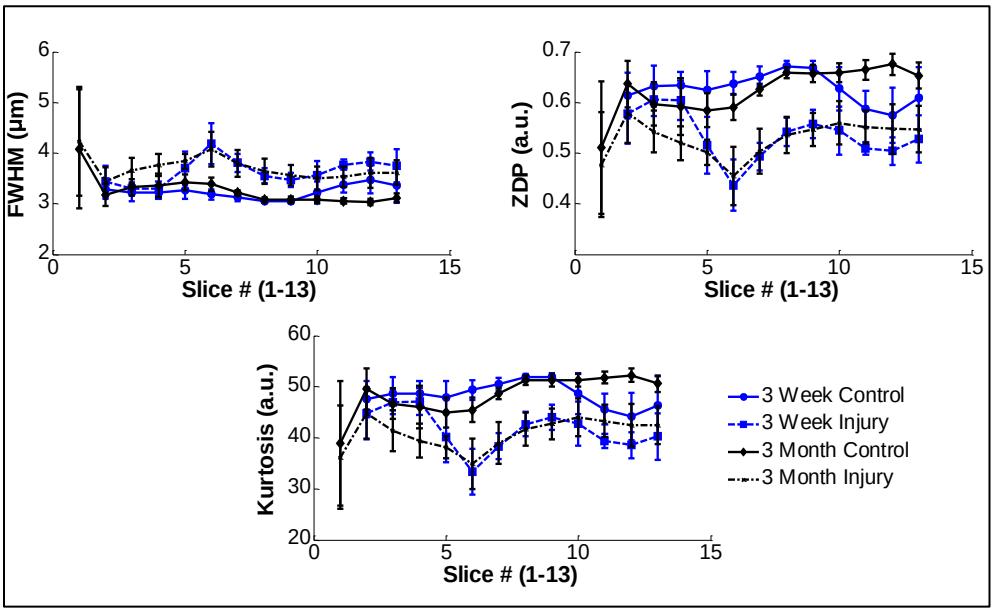


Figure 3. Mean and standard deviation of FWHM, ZDP, and kurtosis values from lateral dorsal ROI averaged over all specimens for each MRI slice position. Three-week values are show in blue and 3-month values in black. Contralateral control ROI values are displayed with solid lines, while injured ROI values are displayed with dashed lines. Slices are numbered in the rostral-caudal direction.

As expected, the injured ROI values show consistently higher FWHM, and lower ZDP and kurtosis values compared with control ROI values. These results suggest degradation in axon morphology. Slice 6 shows the greatest loss of axon integrity based on QSI metrics, which is expected as the site of injury was centered on slice 6.

Conclusion

The results suggest several subtle differences in the degree of loss of axon integrity between the three-week and three-month results. The loss of axon integrity can be inferred from the relative differences between the control and injured ROI values of the QSI parameters. Focusing on the site of injury (slice 6), the results suggest that there is greater axonal injury at three weeks compared with three months post-injury. Also, at three weeks post-injury, there is less evidence of axon degradation rostral (slices 1-5) compared with caudal (slices 7-13) to the site of injury. At three months post-injury, there is evidence of increased loss of axon integrity far away from the site of injury (slices 1-3 and 10-13). Further investigation and comparison with histology is planned, followed by publication of the results in 2015.

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